regulates the threshold of activation of T cells. Our results highlight the functional importance of the cytoskeleton in tuning cellular responses to receptor signals and will be of broad interest to all cell biologists interested in cellular mechanotransduction.

**890-Pos Board B645**

**Combined Clsm and AFM Indentation Reveals Metastatic Cancer Cells Stiffen during Rho/ROCK Contractility-Dependent Invasion of Collagen I Matrices**

**Jack R. Staunton**, Bryant L. Doss, Robert Ros.
Department of Physics & Center for Biological Physics, Arizona State University, Tempe, AZ, USA.

A critical step in the metastatic cascade is the process wherein a single cell breaks through the basement membrane, leaving the primary tumor and entering the stroma whence it can disseminate. In the case of many solid tumor cancers, increased cell deformability is thought to facilitate this process. However, the ligand density, alignment, and stiffness of the matrix determine which mode of motility will succeed. To investigate the mechanical interplay between the cell and ECM during this process, we created a simple model of stromal invasion using 10-200 μm thick bovine collagen I hydrogels ranging from 0.1-5 kPa in Young's modulus that were seeded at low density with highly metastatic MDA-MB-231 breast cancer cells. Significant population fractions invaded the matrices either partially or fully within 24 h. We then combined confocal fluorescence microscopy and AFM indentation to determine the Young's moduli of individual embedded cells and the pericellular matrix using novel analysis methods for heterogeneous samples. In partially embedded cells, we observe a statistically significant correlation between the degree of invasion and the Young's moduli (~220 Pa/μm; $p<0.001$), which was up to an order of magnitude greater than that of the same cells measured in 2D. ROCK inhibition returned the cells' Young's moduli to 2D values (~0.5 kPa) and diminished but did not abrogate invasion. This provides evidence that Rho/ROCK-dependent actomyosin contractility is employed for matrix reorganization during initial invasion, and suggests the observed cell stiffening is due to an attendant increase in actin stress fibers. Yet even with MMP and ROCK inhibition, 39% of cells fully invaded the matrix after 6 days, indicating the cells may have alternate motility mechanisms at their disposal.

**891-Pos Board B646**

**Cellular Adhesion - A Key Mechanism for Compartmentalization and Tumor Spreading?**

**Steve Pawlizak**, Anatol Fritsch, Tobias Thalheim, Dave Ahrens, Josef A. Käs.
Soft Matter Physics Division, University of Leipzig, Leipzig, Germany.

Motivated by the success of a novel surgical method taking account of cellular confinement to compartments to resect cervical cancer [1], the impact of compartmentalization on tumor development and spreading comes more and more into focus of today's cancer research [2].

Compartmentalization is a universal and fundamental organization process in which in vivo different cell populations develop in separate confined areas. It has been observed that even tumor cells are confined to their original compartment for a relatively long time before they finally become able to overcome lineage boundaries.

For that reason, the understanding of the mechanical principles underlying this process is of great importance. The differential adhesion hypothesis gives a first explanation by differences in surface tension and adhesiveness of the interacting cells [3]. In this context, we are investigating whether cellular adhesion is in fact a necessary or even sufficient factor to characterize compartmentalization and tumor spreading.

For our studies, we use various cell types, such as healthy and cancerous breast cell lines of different malignancy as well as primary cells from human brain, breast, and cervix carcinoma. A set of different techniques is applied to characterize their mechanical properties and interactions: Cell-cell-adhesion forces are directly measured with a modified atomic force microscope. The "Optical Stretcher" is used for whole cell rheology. In complementary in vitro experiments, the process of cell aggregation and segregation is studied, employing a newly developed setup for long-term observation of droplet cultures. The combination of these techniques will help to shed some new light on the role of cellular adhesion for compartmentalization.

[1] Höckel, Horn et al., Lancet Oncology 10 (7): 683-692 (2009).
[2] Fritsch et al., Nature Physics 6 (10): 730-732 (2010).
[3] Foty, Steinberg, Dev. Biol. 278 (1): 255-263 (2005).

**892-Pos Board B647**

**Physical Limits on Directional Mechanosensing of Amoeboid Crawling Cells**

**Roland Bouffanais**, Xiaoying Zhu.
SUTD, Singapore, Singapore.

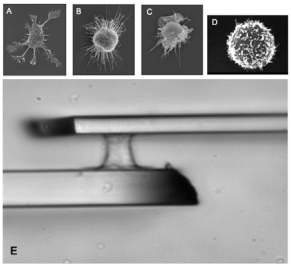
One of the remarkable things about many eukaryotic cells is how effective they are at sensing minute levels of mechanical stimulation, while living in a constantly changing biomechanical environment. In particular, many eukaryotic cells are able to perform directional mechanosensing by directly measuring minute spatial differences in the mechanical stress on their membrane. Using vegetative unpolarized Dictyostelium discoideum amoebae, we report the extremely high directional mechanosensitivity of those cells with mechanostimulus of the order of 0.1 Pa. Based on those experimental evidences, we develop a model to explore the limits of a single mechanosensitive channel activation using a two-state double-well model for the gating mechanism. We then focus on the physical limits of directional mechanosensing by a single cell having multiple mechanosensors and subjected to a shear flow inducing a nonuniform membrane tension similar to what is taking place at the cellular level in our experiment. Our theoretical model demonstrates that the accuracy in sensing the mechanostimulus direction not only increases with cell size and exposure to signal, but also grows for cells with a near-critical membrane prestress. Finally, the existence of a nonlinear threshold effect, fundamentally limiting the cell's ability to effectively perform directional mechanosensing at low signal-to-noise ratio, is reaveled and discussed.

**893-Pos Board B648**

**Mechanical Characterization of Human Monocyte Derived Antigen Presenting Cells**

**Nathalie Bufi**[1], Armelle Bohineust[2], Stéphanie Dogniaux[2], Alain Richert[1], Michael Saitakis[2], Claire Hivroz[2], Atef Asnacios[1].
[1]Laboratoire MSC - CNRS/Paris Diderot, Paris, France, [2]Institut Curie, U932, Paris, France.

T cells are at the root of the immune response protecting living organisms against pathogens and tumor cells, and have recently been shown to respond to mechanical changes. The activation of T cells triggering the immune response requires the formation of cell-cell interaction between T lymphocytes and various antigen presenting cells (APC) (tumor cells, dendritic cells, macrophages, B lymphocytes...). Yet, little is known about the rigidity of these different APCs. In the present study we use the single-cell microplates assay to measure the mechanical properties of human monocyte derived APCs. We show that APCs present a wide range of stiffnesses, that are affected during differentiation, maturation and in response to inflammatory signals.




Noteworthily, dendritic cells, the most efficient APC for T cell activation, present the closest rigidity to T cell rigidity. These results suggest that cell stiffness could be an important factor in immune cell-cell interactions and T cell mediated responses. We will then show preliminary studies on the role of rigidity in T cell activation ; involving laying T cells on substrates mimicking APCs rigidities and monitoring T cell activation and T cell / substrate interface organization.

**894-Pos Board B649**

**Directional Mechanosensing of Amoeboid Cells**

**Xiaoying Zhu**[1], Roland Bouffanais[1], Dick Yue[2].
[1]SUTD, Singapore, Singapore, [2]MIT, Cambridge, MA, USA.

The importance of mechanotactic directional motility has proven to be central in a series of recent experiments involving eukaryotic cells. Hydrodynamic shear stress is one biophysical cue in the extracellular matrix that has been shown to generate compelling mechanotactic behaviors. A microfluidic setup has been designed to generate creeping flows with controllable low-magnitude shear stress and with the possibility to swiftly reverse flow direction within one second. This setup allows us to directly visualize and track the transient responses of multiply seeded cells. Using vegetative Dictyostelium discoideum amoebae, we show that crawling cells have enhanced directional mechanosensitivity with environmental conditions close to those encountered in vivo, in terms of shear stress levels, extracellular calcium